USP purity analysis of pravastatin sodium using the Agilent 1120 Compact LC

Application Note

Manufacturing QA/QC

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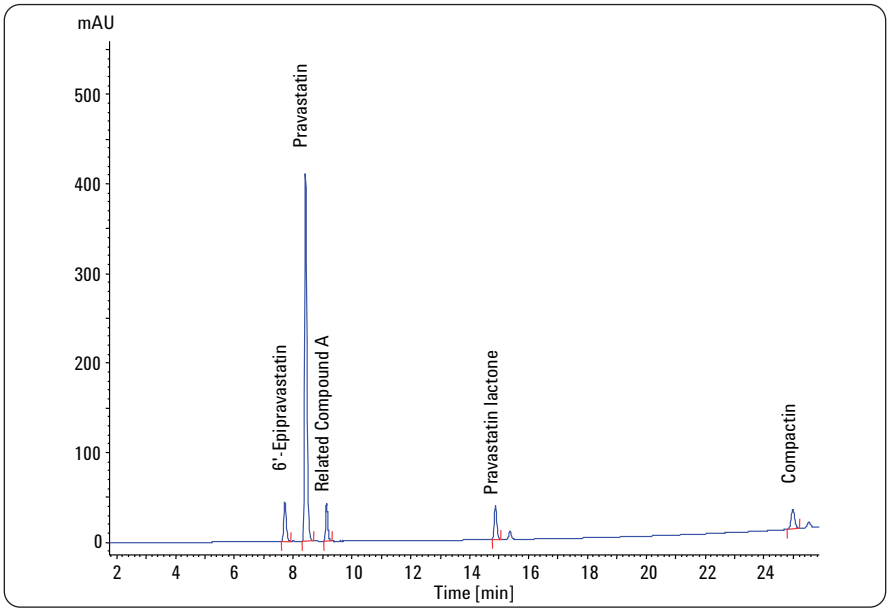

Abstract

Pravastatin sodium helps to reduce cholesterol biosynthesis, thereby preventing cardiovascular disease. The chromatographic method for purity that was established by the United States Pharmacopeia (USP) for pravastatin sodium was performed on the Agilent 1120 Compact LC, and system suitability parameters were verified. The system suitability mixture containing pravastatin 1,1,3,3-tetramethylbutylamine and the impurity known as Related Compound A displayed a resolution of 6.3, which is well above the acceptance limit of not less than 2.0. The detector in the Agilent 1120 Compact LC produced a linear response of Related Compound A down to a concentration of 0.09 µg/mL.

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Introduction

The USP chromatographic method for purity of pravastatin sodium[1] was performed on the Agilent 1120 Compact LC, and system suitability parameters were tested. The system suitability test sample consisted of USP pravastatin 1,1,3,3-tetramethylbutylamine and USP Related Compound A, which is a sodium salt for the impurity 3α-hydroxyisocompactin. Pravastatin sodium and Related Compound A are positional isomers (figure 1).

Pravastatin sodium has six impurities that are reported by USP:

1. 3″-Hydroxypravastatin
2. 6´-Epipravastatin
3. 3α-Hydroxyisocompactin (also called Related Compound A)
4. Pentanoyl impurity
5. Pravastatin lactone
6. Compactin

Only impurities 2, 3, 5, and 6 were used to demonstrate the impurity method on the Agilent 1120 Compact LC.

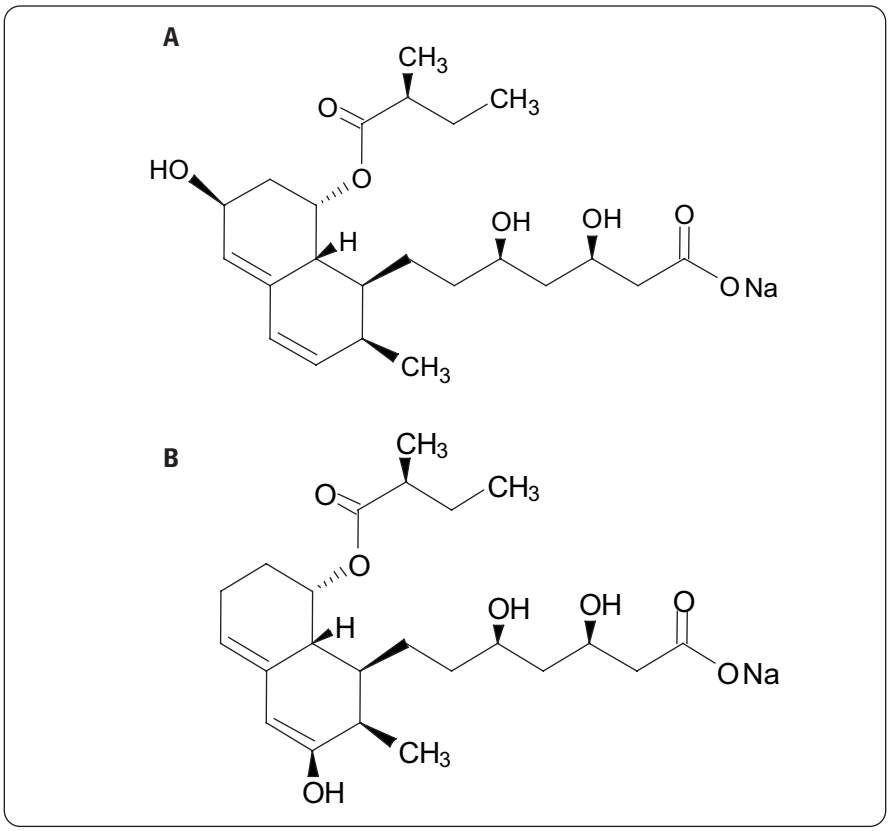

Figure 1
A) Pravastatin sodium. B) USP Related Compound A.

Experimental

Sample preparation

- **Diluent: A** 1:1 mixture of methanol and water was prepared. The methanol was HPLC grade (J.T. Baker). Milli-Q water (Millipore) was used for the experiment.
- **System suitability sample:** Pravastatin 1,1,3,3-tetramethylbutylamine and Related Compound A were dissolved in diluent to obtain a concentration of 0.6 mg of pravastatin 1,1,3,3-tetramethylbutylamine and 0.001 mg of Related Compound A per mL. The system suitability sample was used to determine the value of the relative retention time (RRT) of Related Compound A versus pravastatin. Both pravastatin 1,1,3,3-tetramethylbutylamine and Related Compound A were obtained from the USP.
- **Standard sample:** Pravastatin 1,1,3,3-tetramethylbutylamine was dissolved in diluent to a concentration of 1.25 µg/mL. The standard sample was used to determine the relative standard deviation (RSD) of the peak areas.
- **RRT test sample:** Pravastatin sodium and four of its impurities – 6´-epipravastatin, Related Compound A, pravastatin lactone, and compactin – were dissolved in diluent to a concentration of 65 µg/ml for pravastatin and 7.5 µg/mL for the impurities. The RRT test sample was prepared to determine the RRT of each impurity, for comparison with values reported in the USP. Pravastatin lactone and compactin were obtained from VARDA Biotech. Related Compound A was obtained from the USP and 6´-epipravastatin was obtained from the European Pharmacopeia (EP).
- **Detector linearity test samples:** Seven concentration levels of Related Compound A from 0.09 µg/mL to 1.20 µg/mL were prepared as spiked amounts in the pravastatin sodium sample, which had a constant concentration of 0.5 mg/mL. These samples were used to test the detector response for linearity at low concentrations of the impurity.

Equipment

The Agilent 1120 Compact LC system included:

- A binary pump with integrated degasser
- An autosampler with vial tray
- A variable wavelength detector (VWD)

The instrument was controlled by the Agilent EZChrom Elite Compact software.

Results and Discussion

System suitability results

According to the USP monograph on pravastatin sodium, the system suitability sample containing pravastatin 1,1,3,3-tetramethylbutylamine and Related Compound A should show a resolution of not less than (NLT) 2.0 and Related Compound A should show an RRT of about 1.1. (USP suggests that there are no acceptance criteria applied to RRT, as RRT is an aid in peak identification only.)

Figure 2 shows six replicate injections of the system suitability sample. The results show that Related Compound A has an RRT of 1.1 for all runs and a resolution of 6.3, which is better than the USP limit of NLT 2.0.

Standard deviations of pravastatin peak areas

The standard sample containing 1.25 µg/mL of pravastatin 1,1,3,3-tetramethylbutylamine was injected six times to test the relative standard deviation of the peak areas of pravastatin. According to the USP method, six replicate injections of the standard sample should have a relative standard deviation (RSD) of the peak areas

Parameters	Detail	
Wavelength for VWD	238 nm	
Column	Agilent ZORBAX SB-C18, 4.6 x 75 mm, 3.5 µm	
Diluent	50:50 methanol:water	
Injection volume	10 µL	
Needle wash	Flush port, 3 sec using diluents	
Sample temperature	Ambient	
Column temperature	25 °C	
Mobile phase	Buffer pH 7.0: 0.08 M phosphoric acid solution adjusted with triethylamine to pH 7.0	
Buffer A:	Water:buffer pH 7.0:acetonitrile (52:30:18)	
Buffer B:	Water:buffer pH 7.0:acetonitrile (10:30:60)	
Gradient	Time	% Buffer B
	0	0
	3.0	0
	26.5	100
	26.6	0
	30	0
Post-time	3 min	
Flow	1 mL/min	

Table 1
Instrument parameters.

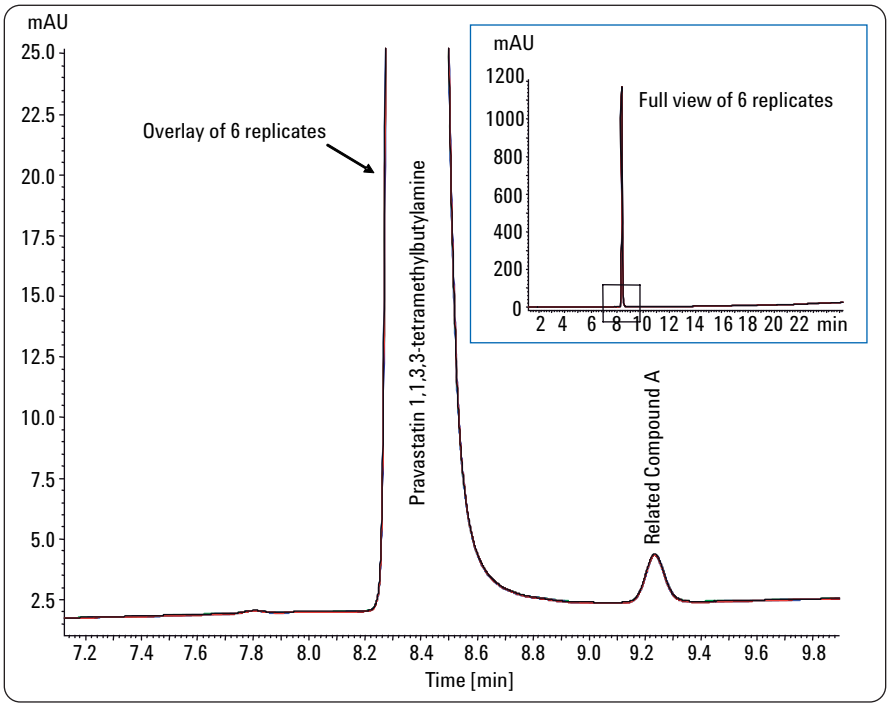

Figure 2
Overlay of six replicate injections of the system suitability sample. The insert shows the full view of the replicate injections.

of not more than (NMT) 10.0 %. A value of 0.3 % RSD was obtained, suggesting the excellent precision of the injector in the Agilent 1120 Compact LC. Figure 3 shows the six injections from the standard sample.

Retention times of the impurities relative to the pravastatin retention time

Figure 4 shows the chromatogram of the RRT test sample analyzed according to the USP test for chromatographic purity for pravastatin. The test was used to calculate RRTs and compare them with the RRTs provided by the USP. Table 2 shows the comparison and excellent match between the RRTs obtained experimentally and those reported by the USP.

Name	USP-reported relative retention time	Experimentally obtained relative retention time
3″-Hydroxypravastatin	0.33	-*
6′-Epipravastatin	0.92	0.91
3α-Hydroxyisocompactin	1.1	1.1
Pentanoyl impurity	1.2	-*
Pravastatin lactone	1.8	1.8
Compactin	3.1	3.1

Table 2
Experimentally determined RRTs of four out of the six impurities reported in the USP method.

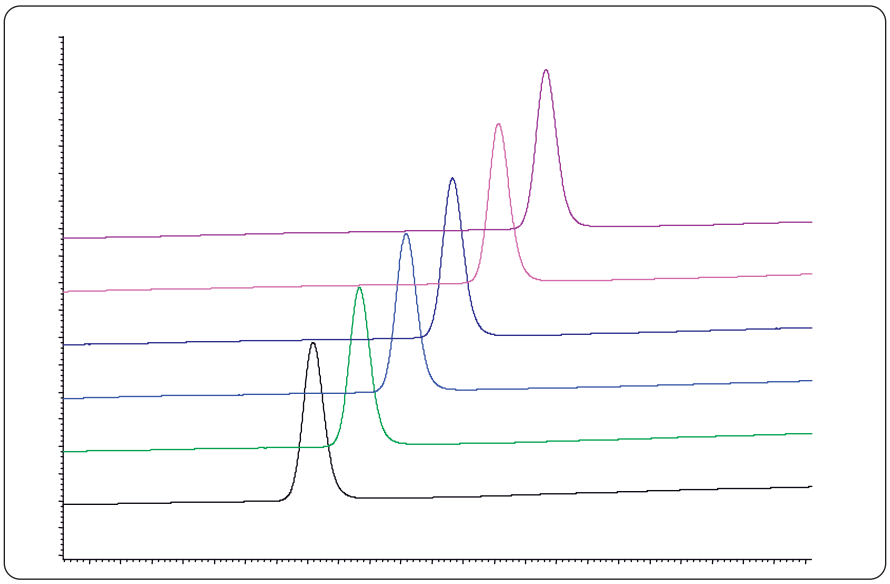

Figure 3
Six replicate injections of the standard sample (x and y axes offset).

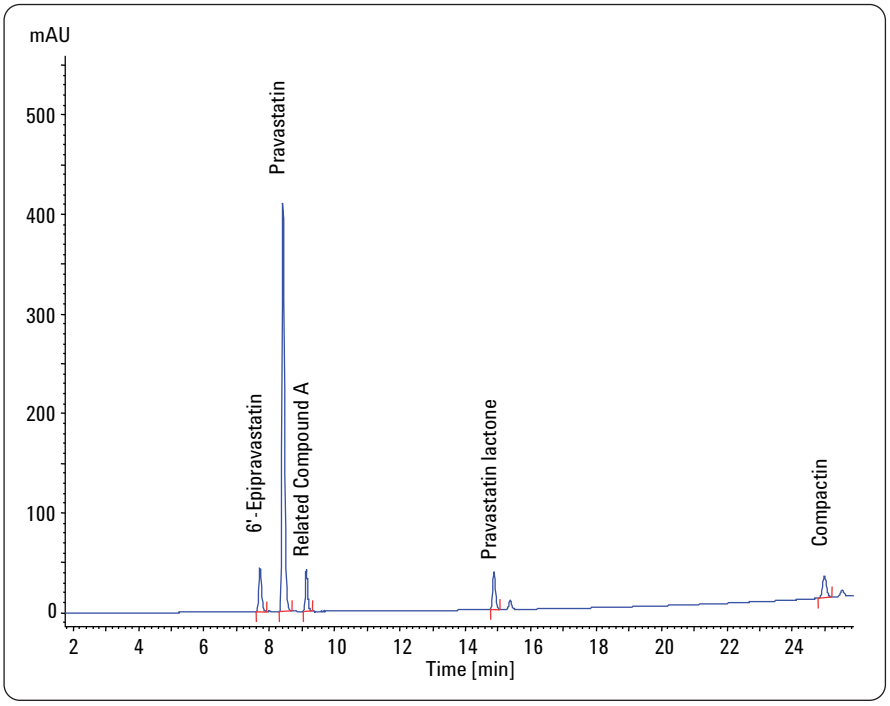

Figure 4
Chromatogram of the RRT test sample. The peaks next to pravastatin lactone and compactin originate from the impurities.

Results of detector linearity tests

The USP method specifies an upper limit concentration of 0.2 % for Related Compound A, which is 1 µg/mL based on a concentration of 0.5 mg/mL for the pravastatin sodium solution. To test the detector's linear response at low levels, 0.09 µg/mL to 1.20 µg/mL of Related Compound A was spiked into 0.5 mg/mL of pravastatin sodium. Table 3 shows signal-to-noise (S/N) ratios obtained at each of the spiked levels. The spiked level with a concentration of 0.17 µg/mL, which corresponds to 0.034 %, is the limit of quantification (LOQ). Figure 5 shows the overlaid chromatograms at each of the injection levels. A uniform rise of the injection amount was seen, demonstrating excellent detector response for the Agilent 1120 Compact LC. The linearity plot of the injection levels is shown in

Level	Related Compound A in µg/mL (% impurity)	S/N ratio (ASTM)	RSD of retention time (n=3)	RSD of area (n=3)
1	0.09 (0.018 %)	6.39	0.02	3.52
2	0.17 (0.034 %)	10.46	0.01	4.15
3	0.33 (0.066 %)	19.38	0.01	0.80
4	0.60 (0.120 %)	33.98	0.01	0.82
5	0.83 (0.166 %)	60.55	0.01	0.62
6	1.03 (0.206 %)	70.18	0.04	0.61
7	1.20 (0.240 %)	81.53	0.03	0.59

Table 3
Related Compound A spiked at levels below and above the limit concentration. The limit concentration was calculated based on a pravastatin concentration of 0.5 mg/mL.

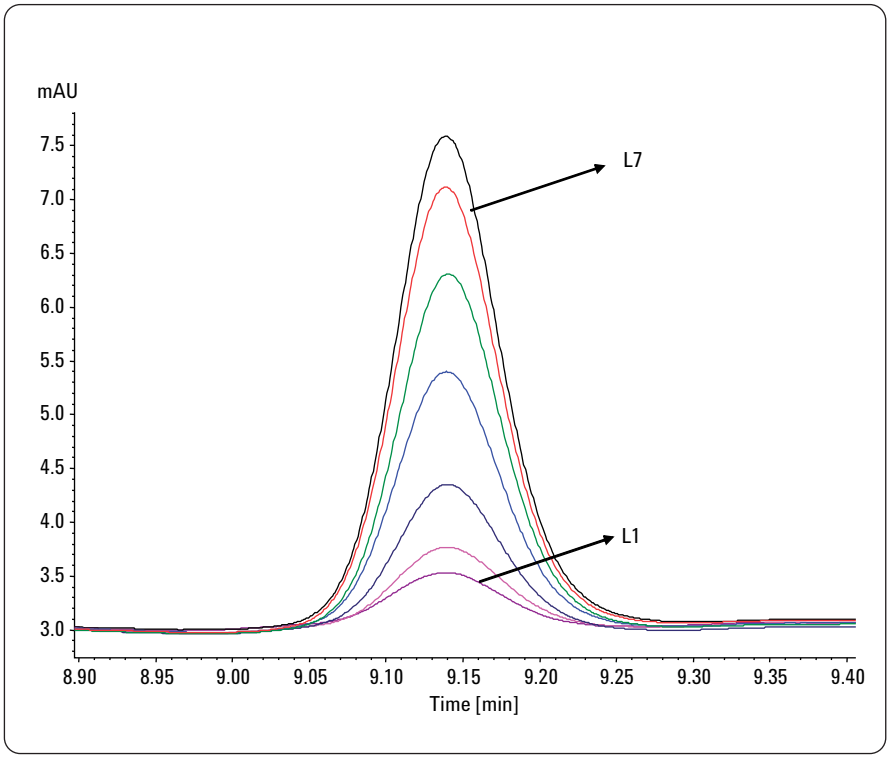

Figure 5
Overlay of the chromatograms for the detector linearity test for Related Compound A. The chromatograms are stacked to display the linear detector response.

Figure 6, where the correlation coefficient (R^2) for the linearity is 0.998.

Conclusion

The method from the USP monograph on chromatographic purity for pravastatin sodium was performed on the Agilent 1120 Compact LC system. The system suitability results showed excellent resolution of 6.3. Injector precision at low concentration levels of pravastatin was demonstrated from replicate injections of the standard sample; an excellent value of 0.3 % was obtained for the RSD of the areas. A linearity value (R^2) of 0.998 was observed for the Related Compound A impurity at levels lower than its limit concentration, demonstrating outstanding detector response.

USP methods are validated methods and do not require revalidation, but do require simple verification of the method. The results of this study clearly show that the USP method was verified on the Agilent 1120 Compact LC.

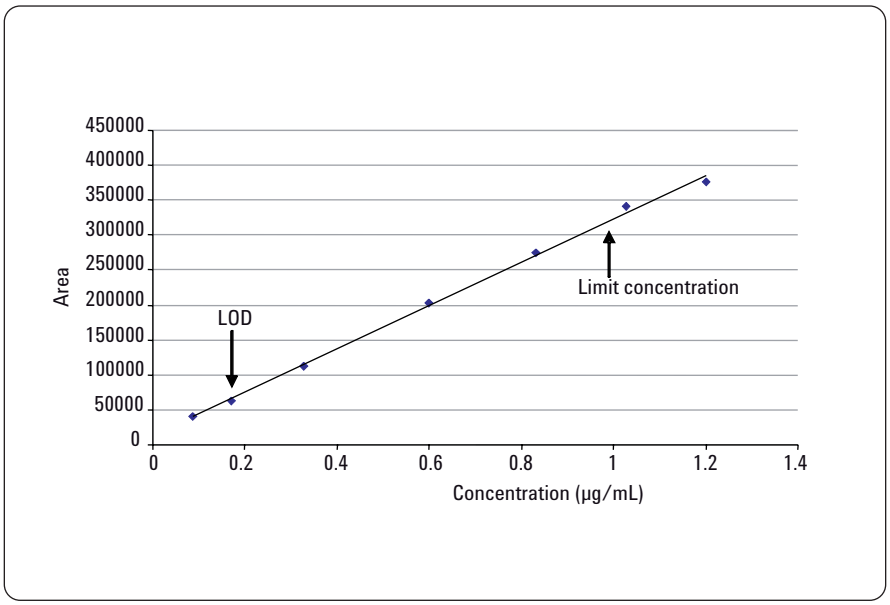

Figure 6
The linearity of the detector response for Related Compound A at spike levels of 0.09 µg/mL to 1.20 µg/mL.

References

1.
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